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p16 immunohistochemistry of multiple primary melanomas as screening to identify Familial Melanoma Syndrome

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The development of more than one primary malignant melanoma (MM) in a patient has been reported to range from 1.3 to 8.2%.¹ The multiple primary melanomas (MPM) represent a valid study model for clinical and biomolecular characterization of Familial Melanoma Syndrome. Hereditary MM is the presence of MM in at least 2 first-degree relatives or in three relatives independent of degree.² The pathogenesis of these inherited conditions is linked to germline cyclin-dependent kinase inhibitor 2A (CDKN2A) mutations.^{3–5} Among preliminary molecular screening tests, the role of immunohistochemical (IHC) analysis, particularly for p16 protein, is debated. Further studies are needed to confirm that IHC analysis is a suitable modality to screen for CDKN2A abnormalities in paraffin-embedded human MM tissue.

In this study, we evaluated the efficacy of p16 IHC analysis of MPM as preliminary screening to ascertain hereditary setting. We studied all patients with a diagnosis of at least two synchronous or metachronous primary MMs identified by a melanoma registry. Detailed family histories were collected for each MPM patient. Immunohistochemistry of p16, MLH1, MSH2, beta-catenin, E-cadherin and p53 proteins was performed on paraffin-embedded tumor samples. Altered expression of p16 was considered when loss of nuclear expression was observed.^{6,7} Analysis of germline CDKN2A gene mutations was performed on peripheral blood mononuclear cells of patients.⁸ Deletions of 9p21 locus were carried out on paraffin-embedded tumor samples using the fluorescence *in situ* hybridization method.

A total of 27 unrelated patients were identified who had been diagnosed with at least two primary MMs. Of these, 25 presented two lesions and two patients presented three lesions. The first two MMs were synchronous in nine patients; in those that were not synchronous, the median interval between the first two MMs was 18 months (range: 0–36 months). A careful evaluation of family history identified a positive MM history in nine of 27 (36.0%) patients and seven had at least 1 first-degree relative affected by MM, corresponding to the clinical criteria for definition of Familial MM. Among the MPM familial cases, a family with four patients having MMs (two of whom had two primary MMs) and one patient with pancreatic cancer was identified (Fig. 1).

The main IHC result was that, in the MMs of three MPM patients, lack of p16 nuclear expression – with weak cytoplasmic expression – was observed (Fig. 2b). No significant association was found between p53 expression and other clinicopathologic variables as well as for β -catenin and E-cadherin expression. p53 protein was overexpressed in four cases and in two of these the overexpression was seen in both MMs of the same patient. Lack of expression of E-cadherin was shown in three patients. Nuclear expression of β -catenin was present in two cases in addition to a membranous pattern and in one case with prevalent nuclear localization. In three cases, we found a coexistence of several protein expression

alterations, in particular, adhesion molecules, β -catenin and E-cadherin, with p53 and p16 proteins (Table 1).

Germline CDKN2A mutation (T to C transition at bp 194 of exon 2, which results in a missense mutation L65p in p16 and in a silent mutation A79H in p14^{ARF}) was identified in a MPM proband of a patient with Familial Melanoma Syndrome with lack of p16 nuclear expression (Fig. 1). 9P21 large deletion was identified in the two other MPM patients with positive family history for whom lack of p16 nuclear expression was detected at IHC analysis of MM.

Our clinical results indicate that more than 30% of MPM patients have a positive family history of MMs. We believe that our higher rate of familial cases among MPM patients is accurate as it was determined through a very detailed family history reconstruction including first-, second- and third-degree relatives.⁹ Increased MM risk is present in relatives of MPM patients so that screening programs and counseling may be offered to them. The collection of detailed family histories for the reconstruction of an accurate genealogic tree is crucial for familial lifetime risk evaluation.

The role of IHC evaluation enables us to suggest an approach such as preliminary molecular screening for all MPM patients. Hereditary MMs from patients with p16 germline mutations show disappearance of staining from the nucleus, and weak immunoreactivity in the cytoplasm (Fig. 2). Different mechanisms of p16 inactivation (promoter methylation, 9P21 large deletion, etc.) could be responsible for this staining pattern.^{10–12} Our IHC evidence revealed a concordance between lack of p16 nuclear expression and biomolecular evidence of CDKN2A aberrations (both L65P germline mutations and 9P21 large deletions).

We have shown that, in all MPM patients, p16 IHC analysis should be adopted for the selection of cases that may benefit from direct sequencing analysis of the CDKN2A gene. The combined use of clinical features and p16 IHC analysis can be useful for characterization of these multiple primary MM patients and recognition of the hereditary setting.

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Figure 1.
Genealogic tree of familial multiple primary melanomas patients. Numbers in parentheses indicate each patient's age at diagnosis

Figure 2.

(a) Nuclear p16 expression in malignant melanoma; (b) Lack of nuclear p16 expression with weak cytoplasmic expression in hereditary multiple primary melanomas: (a) $\times 200$; (b) $\times 200$

Table 1

Clinical and molecular features of MPM patients

No.	Sex	Age (years)	MM thickness (mm)	Clark's Level	Site	IHC					CDKN2A/ Del 9p21	MSH2	MLH1	E-cadherin	β-catenin	p53	p16	Other tumors	Tumors in family
						p16	p53	β-catenin	E-cadherin	MLH1	MSH2								
1	M	93	5	IV	Nose	N	+	Focal	M	N	N	N	N	N	N	N	N	Basocellular epithelioma	
2	F	94	0.92	II	Nose tip*	N	+	Focal	M	N	N	N	N	N	N	N	N		
		39	0.5	III	Abdomen	N	-	M	M	N	N	N	N	N	N	N	N	Colonic adenomas	
3	F	39	1.26	III	Left leg	N	+	M	Absent	N	N	N	N	N	N	N	N		
		52	0.49	II	Left shoulder-blame	N	+	M	M	N	N	N	N	N	N	N	N		
4	F	65	0.59	III	Right leg	N	-	M	M	N	N	N	N	N	N	N	N		
		65	0.36	II	Left leg (<i>ex nevus</i>)	N	-	M	M	N	N	N	N	N	N	N	N		
5	M	30	1.8	II	Left buttock	N				N	N	N	N	N	N	N	N		
		33	0.3	II	Left upper limb	N	-	M	M	N	N	N	N	N	N	N	N		
6	F	67	0.3	II	Right cheek	N	-	M	M	N	N	N	N	N	N	N	N		MM Brother
		67	1.44	III	Right temple*	N	-	M	M	N	N	N	N	N	N	N	N		
7	F	63	Hypodermic	V	Left leg	N	-	M	M	N	N	N	N	N	N	N	N	9p21 deletion	MM Daughter
		67	1.65	III	Left leg	Lack of nuclear p16	-	M	M	N	N	N	N	N	N	N	N		
8	M	32	0.44	II	Lumbar region	N	-	M	M	N	N	N	N	N	N	N	N		MM Aunt
		34	<i>In situ</i>		Right shoulder	N	-	M	M	N	N	N	N	N	N	N	N		
9	M	65	1.15	IV	Abdomen	N	-	M	M	N	N	N	N	N	N	N	N		
		66	0.35	II	Left shoulder	N	-	M	M	N	N	N	N	N	N	N	N		
10	M	35	0.6	II	Back	N	-	N	M	N	N	N	N	N	N	N	N		MM Mother
		36	0.65	II	Sacral region	N	-	N	M	N	N	N	N	N	N	N	N		
11	F	70	0.28	II	Back (<i>ex nevus</i>)	N	-	M	M	N	N	N	N	N	N	N	N		
		70	0.47	II	Left cervical region	N	-	M	M	N	N	N	N	N	N	N	N		
11	F	59	1.2	III	Right ankle	N	-	M	M	N	N	N	N	N	N	N	N		

IHC														
No.	Sex	Age (years)	MM thickness (mm)	Clark's Level	Site	p16	p53	β-catenin	E-cadherin	MLH1	MSH2	CDKN2A/ Del 9p21	Other tumors	Tumors in family
12	M	61	1.73	IV	Right leg	N	–	M	Absent	N	N			
		59	0.75	II	Back	N	–	M	M	N	N			
		59	1.15	III	Hip	N	–	M	M	N	N			
		60	0.77		Scalp									
13	M	68	1	III	Left forearm	N	–	M	M	N	N		Gist	MM Brother
		68	<i>In situ</i>		Left leg (<i>ex nevi</i>)	N	–	M	M	N	N			
14	F	24	0.65	II	Back (<i>ex nevi</i>)	N	–	M	M	N	N			
		25	0.21	II	Back (<i>ex nevi</i>)	N	–	M	M	N	N			
15	M	74	2.51	IV	Left side of back	N	+	M	M	N	N			
		74	0.44	II	Chest	N	–	M	M	N	N			
		46	1.21	IV	Right shoulder-blade	N	–	M	M	N	N			
16	F	46	0.46	II	Right paravertebral region	N	–	M	M	N	N			
		53	0.25	II	Back (<i>ex nevi</i>)	N	–	M	M	N	N			
17	M	54	1.07	III	Right axilla	N	–	M	M	N	N			
		68	0.44	II	Left cheek	N	–	M	M	N	N		Urothelial carcinoma	
18	M	69	0.14	II	Back	N	–	M	M	N	N			
		50	<i>In situ</i>		Forehead	N	–	M	M	N	N		Bowen Syndrome	MM Son
19	F	60	0.22	II	Forehead left upper side	N	–	M	M	N	N			
		39	2.77	IV	Right lumbar region	Lack of nuclear p16	–	M	M	N	N	Mut. L65P	Pancreas	MM Mother
20	M	39	<i>In situ</i>		Right shoulder		–	M	M	N	N			MM Brother
			<i>In situ</i>		Nape (<i>ex nevi</i>)									MM Uncle
21	M	54	0.72	IV	Left thigh	N	–	M	M	N	N			
		55	0.39	II	Back	N	–	M	M	N	N			
22	F	21	0.3	II	Thigh	N	–	M	M	N	N			

IHC														
No.	Sex	Age (years)	MM thickness (mm)	Clark's Level	Site	p16	p53	β-catenin	E-cadherin	MLH1	MSH2	CDKN2A/ Del 9p21	Other tumors	Tumors in family
23	M	32	<i>In situ</i>		Right hip	N	–	M	M	N	N			
		37	0.7	III	Abdomen	N	–	Focal		N	N			
		40	0.39	II	Left shoulder	N	–	M	M	N	N			
24	F	36	2.82	IV	Abdomen (<i>ex nevi</i>)	Lack of nuclear p16	+	M	Absent	N	N	9p21		MM Uncle
25	M	36	<i>In situ</i>		Right shoulder		–	M	M	N	N		deletion	
		31	1.88	IV	Ear	N	–	M	M	N	N			
		33	0.2	II	Back	N	–	M	M	N	N			
26	M	26	0.2	II	Left thigh	N	–	M						
		36	<i>In situ</i>		Right side of chest (<i>ex nevi</i>)	N	–	M						
27	M	36	0.23	II	Right thigh	N	–	M						
		67	0.22	II	Right shoulder-blame	N	–	M	M	N	N			MM Son
		69	–	–	Choroid	N	–	M	M	N	N		Ocular MM	

IHC: Immunohistochemistry analysis; N: nuclear expression; M: membranous expression; MM: malignant melanoma.